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(54) **Combinations of hepatitis C virus (HCV) antigens for use in immunoassays for anti-HCV antibodies**

Kombinationen Hepatitis-C-Virus(HCV)-Antigene zur Anwendung in Immunoassays für Anti-HCV-Antikörper

Combinaisons d'antigènes de l'hépatitis C virus (HCV) pour usage dans des échantillons immunologiques pour anticorps anti-HCV

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Description

Technical Field

The present invention is in the field of immunoassays for HCV (previously called Non-A, Non-B hepatitis virus). More particularly, it concerns combinations of HCV antigens that permit broad range immunoassays for anti-HCV antibodies.

Background

The disease known previously as Non-A, Non-B hepatitis (NANBH) was considered to be a transmissible disease or family of diseases that were believed to be viral-induced, and that were distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH was due to a transmissible infectious agent or agents. Epidemiologic evidence suggested that there may be three types of NANBH: a water-borne epidemic type; a blood-borne or parenterally transmitted type; and a sporadically occurring (community acquired) type. However, until recently, no transmissible agent responsible for NANBH had been identified, and clinical diagnosis and identification of NANBH had been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies were agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

In 1987, scientists at Chiron Corporation (the owner of the present application) identified the first nucleic acid definitively linked to blood-borne NANBH. See, e.g., EPO Pub. No. 318,216: Houghton *et* al., Science 244:359 (1989). These publications describe the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1". HCV is a Flavi-like virus, with an RNA genome.

US Patent 5,350,671 (Houghton et *al*), incorporated herein by reference, describes the preparation of various recombinant HCV polypeptides by expressing HCV cDNA and the screening of those polypeptides for immunological reactivity with sera from HCV patients. That limited screening showed that at least five of the polypeptides tested were very immunogenic; specifically, those identified as 5-1-1, C100, C33c, CA279a, and CA290a. Of these five polypeptides, 5-1-1 is located in the putative NS4 domain; C100 spans the putative NS3 and NS4 domains; C33c is located within the putative NS3 domain and CA279a and CA290a are located within the putative C domain. The screening also showed that no single polypeptide tested was immunologically reactive with all sera. Thus, improved tests, which react with all or more samples from HCV positive individuals, are desirable.

EP-A-445,423, filed on 22nd December 1990 and published on 11th September 1991 describes immunoassays for HCV. EP-A-445,423 describes the use of the C100-3 recombinant yeast/hepatitis C virus SOD fusion polypeptide (disclosed in EP-A-318,216) together with a polypeptide selected from the group consisting of, *inter alia,* p1, p35 and p99. The peptide p1 corresponds to amino acids residues 1 to 75 of Figure 1A (where position 9 is Lys and 11 is Asn), p35 corresponds to amino acid residues 35 to 75 of Figure 1A, and p99 corresponds to residues 99 to 126 of Figure 1A.

WO91/15574, published on 17 October 1991 describes, *inter alia,* purified proteins and glycopeptides of HCV useful in a diagnostic system for detection of human HCV antisera. EP-A-442 394 describes synthetic peptides for the detection of antibodies to HCV.

Disclosure of the Invention

Applicants have carried out additional serological studies on HCV antigens that confirm that no single HCV polypeptide identified to date is immunologically reactive with all sera. This lack of a single polypeptide that is universally reactive with all sera from individuals with HCV may be due, *inter alia,* to strain-to-strain variation in HCV epitopes, variability in the humoral response from individual-to-individual and/or variation in serology with the state of the disease.

These additional studies have also enabled applicants to identify combinations of HCV antigens that provide more efficient detection of HCV antibodies than any single HCV polypeptide.

Accordingly, one aspect of this invention is a combination of hepatitis C virus (HCV) epitope sequences in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

(a) a first epitope sequence from the C domain of the HCV polyprotein;

(b) a second epitope sequence from a second domain of the HCV polyprotein which domain is:

(i) the NS3 domain of the HCV polyprotein;
(ii) the NS4 domain of the HCV polyprotein; or
(iii) the NS5 domain of the HCV polyprotein;

and

(c) a third epitope sequence from a third domain of the HCV polyprotein which domain is:

(i) the NS3 domain of the HCV polyprotein;
(ii) the NS4 domain of the HCV polyprotein; or
(iii) the NS5 domain of the HCV polyprotein;

wherein the third domain is different from the second domain; with the proviso that the combination is not the peptide pI with C100-3, the peptide p35 with C100-3 or the peptide p99 with C100-3.

In one embodiment, the combination of HCV epitope sequences is in the form of a fusion protein comprised of the epitopes. In an alternative embodiment, the combination of epitope sequences is in the form of the individual epitopes bound to a common solid matrix. In still another embodiment, the combination of epitope sequences is in the form of a mixture of the individual epitopes.

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the above-described combination of HCV epitope sequences under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said epitope sequences.

Said body component may be contacted with a panel of HCV epitope sequences simultaneously or sequentially.

Another aspect of the invention is a kit for carrying out an assay for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising in packaged combination

(a) said combination of HCV epitope sequences;
(b) standard control reagents; and
(c) instructions for carrying out the assay.

Brief Description of the Drawings

In the drawings:

Figure 1 is the nucleotide sequence of the cDNA sense and anti-sense strand for the HCV polyprotein and the amino acid sequence encoded by the sense strand.

Figure 2 is a schematic of the amino acid sequence of Figure 1 showing the putative domains of the HCV polypeptide.

Modes for Carrying Out the Invention

Definitions

"HCV antigen" means a polypeptide of at least about 5 amino acids, more usually at least about 8 to 10 amino acids that defines an epitope found in an isolate of HCV. Preferably, the epitope is unique to HCV. When an antigen is designated by an alphanumeric code, the epitope is from the HCV domain specified by the alphanumeric.

"Synthetic" as used to characterize an HCV antigen means that the HCV antigen has been man-made such as by chemical or recombinant synthesis.

"Domains" means those segments of the HCV polyprotein shown in Figure 2 which generally correspond to the putative structural and nonstructural proteins of HCV. Domain designations generally follow the convention used to name Flaviviral proteins. The locations of the domains shown in Figure 2 are only approximate. The designations "NS" denotes "nonstructural" domains, while "S" denotes the envelope domain, and "C" denotes the nucleocapsid or core domain.

"Fusion polypeptide" means a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

"Common solid matrix" means a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

"Mammalian body component" means a fluid or tissue of a mammalian individual (e.g., a human) that commonly contains antibodies produced by the individual. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Immunologically reactive" means that the antigen in question will react specifically with anti-HCV antibody commonly present in a significant proportion of sera from individuals infected with HCV.

"Immune complex" means the combination or aggregate formed when an antibody binds to an epitope on an antigen.

Combinations of HCV Antigens

Figure 2 shows the putative domains of the HCV polyprotein. The domains from which the antigens used in the combinations derive are: C, S (or E), NS3, NS4, and NS5. The C domain is believed to define the nucleocapsid protein of HCV. It extends from the N-terminal of the polyprotein to approximately amino acid 120 of Figure 1. The S domain is believed to define the virion envelope protein, and possibly the matrix (M) protein, and is believed to extend from approximately amino acid 120 to amino acid 400 of Figure 1. The NS3 domain extends from approximately amino acid 1050 to amino acid 1640 and is believed to constitute the viral protease. The NS4 domain extends from the terminus of NS3 to approximately amino acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about amino acid 2000 to the end of the polyprotein and is believed to define the viral polymerase.

The sequence shown in Figure 1 is the sequence of the HCV1 isolate. It is expected that the sequences of other strains of the blood-borne HCV may differ from the sequence of Figure 1, particularly in the envelope (S) and nucleocapsid (C) domains. The use of HCV antigens having such differing sequences is intended to be within the scope of the present invention, provided, however, that the variation does not significantly degrade the immunological reactivity of the antigen to sera from persons infected with HCV.

In general, the HCV antigens will comprise entire or truncated domains, the domain fragments being readily screened for antigenicity by those skilled in the art. The individual HCV antigens used in the combination will preferably comprise the immunodominant portion (i.e., the portion primarily responsible for the immunological reactivity of the polypeptide) of the stated domain. In the case of the C domain it is preferred that the C domain antigen comprise a majority of the entire sequence of the domain. The antigen designated C22 (see Example 4, infra), is particularly preferred. The S domain antigen preferably includes the hydrophobic subdomain at the N-terminal end of the domain. This hydrophobic subdomain extends from approximately amino acid 199 to amino acid 328 of Figure 1. The HCV antigen designated S2 (see Example 3, infra), is particularly preferred. Sequence downstream of the hydrophobic subdomain may be included in the S domain antigen if desired.

A preferred NS3 domain antigen is the antigen designated C33c. That antigen includes amino acids 1192 to 1457 of Figure 1. A preferred NS4 antigen is C100 which comprises amino acids 1569 to 1931 of Figure 1. A preferred NS5 antigen comprises amino acids 2054 to 2464 of Figure 1.

The HCV antigen may be in the form of a polypeptide composed entirely of HCV amino acid sequence or it may contain sequence exogenous to HCV (i.e., it may be in the form of a fusion protein that includes exogenous sequence). In the case of recombinantly produced HCV antigen, producing the antigen as a fusion protein such as with SOD, alpha-factor or ubiquitin (see commonly owned U.S. Pat. No. 4,751,180, U.S. Pat. No. 4,870,008 and U.S. Pat. Application Serial. No. 390,599, filed 7 August 1989, which describe expression of SOD, alpha-factor and ubiquitin fusion proteins) may increase the level of expression and/ or increase the water solubility of the antigen. Fusion proteins such as the alpha-factor and ubiquitin fusion are processed by the expression host to remove the heterologous sequence. Alpha-factor is a secretion system, however, while ubiquitin fusions remain in the cytoplasm.

Further, the combination of antigens may be produced as a fusion protein. For instance, a continuous fragment of DNA encoding C22 and C33c may be constructed, cloned into an expression vector and used to express a fusion protein of C22 and C33c. In a similar manner fusion proteins of C22 and C100; C22 and S2; C22 and an NS5 antigen; C22, C33c, and S2; C22, C100 and S2, and C22, C33c, C100, and S2 may be made. Alternative fragments from the exemplified domain may also be used.

Preparation of HCV Antigens

The HCV antigens of the invention are preferably produced recombinantly or by known solid phase chemical synthesis. They may, however, also be isolated from dissociated HCV or HCV particles using affinity chromatography techniques employing antibodies to the antigens.

When produced by recombinant techniques, standard procedures for constructing DNA encoding the antigen, cloning that DNA into expression vectors, transforming host cells such as bacteria, yeast, insect, or mammalian cells, and expressing such DNA to produce the antigen may be employed. As indicated previously, it may be desirable to

express the antigen as a fusion protein to enhance expression, facilitate purification, or enhance solubility. Examples of specific procedures for producing representative HCV antigens are described in the Examples, infra, and in US patent 5,350,671.

Formulation of Antigens for Use in Immunoassay

The HCV antigens may be combined by producing them in the form of a fusion protein composed of two or more of the antigens, by immobilizing them individually on a common solid matrix, or by physically mixing them. Fusion proteins of the antigen may also be immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface will vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface will be the wall of the well or cup. For assays using beads, the solid surface will be the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface will be the surface of the material from which the dipstick is made. In agglutination assays the solid surface may be the surface of latex or gelatin particles. When individual antigens are bound to the matrix they may be distributed homogeneously on the surface or distributed thereon in a pattern, such as bands so that a pattern of antigen binding may be discerned.

Simple mixtures of the antigens comprise the antigens in any suitable solvent or dispersing medium.

Assay Formats Using Combinations of Antigens

The HCV antigens may be employed in virtually any assay format that employs a known antigen to detect antibodies. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radio-active, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogenous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidine fluoride (known as Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immulon™ 1 or Immulon™ 2 microtiter plates or 6.4 mm (0.25 inch) polysterene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptides is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

In a homogeneous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

In a standard format, the amount of HCV antibodies forming the antibody-antigen complex is directly monitored. This may be accomplished by determining whether labeled anti-xenogenic (e.g., anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format, the amount of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

Complexes formed comprising anti-HCV antibody (or, in the case of competetive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled HCV antibodies in the complex may be detected using a conjugate of antixenogeneic Ig complexed with a label, (e.g., an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the HCV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of precipitate. If no anti-HCV antibody is present in the test specimen, no visible precipitate is formed.

The HCV antigens will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the combination of antigens (either already bound to a solid matrix or separate with reagents for binding them to the matrix), control antibody formulations (positive and/or negative), labeled antibody when the assay format requires same and signal generating reagents (e.g., enzyme substrate) if the label does not

generate a signal directly. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay usually will be included in the kit.

The following examples are intended to illustrate the invention and are not intended to limit the invention in any manner.

Example 1: Synthesis of HCV Antigen C33c

HCV antigen C33c contains a sequence from the NS3 domain. Specifically, it includes amino acids 1192-1457 of Figure 1. This antigen was produced in bacteria as a fusion protein with human superoxide dismutase (SOD) as follows. The vector pSODcfl (Steiner et al. (1986), J. Virol. 58:9) was digested to completion with EcoRI and BamHI and the resulting EcoRI,BamHI fragment was ligated to the following linker to form pcflEF:

GATC CTG GAA TTC TGA TAA
GAC CTT AAG ACT ATT TTA A

A cDNA clone encoding amino acids 1192-1457 and having EcoRI ends was inserted into pcf1EF to form pcf1EF/C33c. This expression construct was transformed into D1210 E. coli cells.

The transformants were used to express a fusion protein comprised of SOD at the N-terminus and in-frame C33c HCV antigen at the C-terminus. Expression was accomplished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of the transformants. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000 x g at 4°C for 20 minutes. The packed cells can be stored at -80°C for several months.

In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD-C33c was isolated and subjected to differential extraction with an alkaline-NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose(TM) and Q-sepharose(TM).

The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000 x g for 15 min at 4°C. After the supernatant was removed, the cell pellets were resuspended in 10 ml of Buffer Al (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM betamercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4,000 x g for 15 minutes at 4°C. After removal of the clear supernatant (periplasmic fraction I), the cell pellets were resuspended in Buffer Al, incubated on ice for 10 minutes, and again centrifuged at 4,000 x g for 15 minutes at 4°C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5 ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter)(obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

The insoluble fraction of the crude extract was collected by centrifugation at 20,000 x g for 15 min at 4°C, washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q(TM)water.

A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging it 20,000 x g for 20 min at 4°C, and retaining the supernatant.

In order to purify SOD-C33c on S-Sepharose(TM), the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose(TM) Fast Flow (1.5 x 10 cm) which had been equilibrated with Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA). After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected.- The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order to identify fractions containing SOD-C33c, aliquots of the fractions were analyzed by electrophoresis on 10% polyacrylamide gels containing SDS followed by staining with Coomassie blue. The fractions are also analyzable by Western blots using an antibody directed against SOD. Fractions containing SOD-C33c were pooled.

Further purification of SOD-C33c was on a Q-Sepharose(TM) column (1.5 x 5 cm) which was equilibrated with Buffer B. The pooled fractions containing SOD-C33c obtained from chromatography on S-Sepharose(TM) was applied to the column. The column was then washed with Buffer B, and eluted with 60 ml of a gradient of 0.0 to 0.4 M Nacl in Buffer B. The flow rate for application, wash, and elution was 1 ml/min; collected fractions were 1 ml. All fractions from

the Q-Sepharose(TM) column were analyzed as described for the S-Sepharose(TM) column. The peak of SOD-C33c eluted from the column at about 0.2 M NaCl.

The SOD-C33c obtained from the Q-Sepharose(TM) column was greater than about 90% pure, as judged by analysis on the polyacrylamide SDS gels and immunoblot using a monoclonal antibody directed against human SOD.

Example 2: Synthesis of HCV Antigen C100

HCV antigen C100 contains sequences from the NS3 and NS4 domains. Specifically, it includes amino acids 1569-1931 of Figure 1. This antigen was produced in yeast. A cDNA fragment of a 1270 bp encoding the above amino acids and heaving EcoRI termini was prepared.

The construction of a yeast expression vector in which this fragment was fused directly to the S. cerevisiae ADH2/GAP promoter was accomplished by a protocol which included amplification of the C100 sequence using a PCR method, followed by ligation of the amplified sequence into a cloning vector. After cloning, the C100 sequence was excised, and with a sequence which contained the ADH2/GAP promoter, was ligated to a large fragment of a yeast vector to yield a yeast expression vector.

The PCR amplification of C100 was performed using as template the vector pS3-56$_{C100m}$, which had been linearized by digestion with SalI. pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dismutase gene, and a downstream alpha factor transcription terminator.

The oligonucleotide primers used for the amplification were designed to facilitate cloning into the expression vector, and to introduce a translation termination codon. Specifically, novel 5'-HindIII and 3'-SalI sites were generated with the PCR oligonucleotides. The oligonucleotide containing the SalI site also encodes the double termination codons, TAA and TGA. The oligonucleotide containing the HindIII site also contains an untranslated leader sequence derived from the pgap63 gene, situated immediately upstream of the AUG codon. The pEco63GAPDH gene is described by Holland and Holland (1980) and by Kniskern et al. (1986). The PCR primer sequences used for the direct expression of C100m were:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG CTC
ACT TTC TAT CCC AGA CAA AGC AGA GT 3'

and

5' GAG TGC TCG TCG ACT CAT TAG GGG GAA
ACA TGG TTC CCC CGG GAG GCG AA 3'.

Amplification by PCR, utilizing the primers, and template, was with a Cetus-Perkin-Elmer PCR kit, and was performed according to the manufacturer's directions. The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes; and the final incubation was at 72°C for 10 minutes. The DNA can be stored at 4°C or -20°C overnight.

After amplification, the PCR products were digested with HindIII and SalI. The major product of 1.1 kb was purified by electrophoresis on a gel, and the eluted purified product was ligated with a large SalI-HindIII fragment of pBR322. In order to isolate correct recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII- SalI fragments excised from the clones. One of the clones which contained the a HindIII-SalI fragment of the correct size was named pBR322/C100-d. Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-SalI fragment.

The expression vector containing C100 was constructed by ligating the HindIII-SalI fragment from pBR322/C100-d to a 13.1 kb BamHI-SalI fragment of pBS24.1, and a 1369 bm BamHI-HindIII fragment containing the ADH2/ GAP promoter. (The latter fragment is described in EPO 164,556). The ADH2/GAP promoter fragment was obtained by digestion of the vector pPGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

Competent HB101 cells were transformed with the recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and SalI digestion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100-d#3.

In order to express C100, competent cells of Saccharomyces cerevisiae strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prc1-407[cir-0]) were transformed with the expression vector pC100-d#3. The transformed cells were plated on URA-sorbitol, and individual transformants were then streaked on Leu$^-$ plates.

Individual clones were cultured in Leu$^-$, ura$^-$ medium with 2% glucose at 30°C for 24-36 hours. One liter of Yeast

Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight culture, and the resulting culture was grown at 30°C at an agitation rate of 400 rpm and an aeration rate of 1 L of air per 1 L of medium per minute (i.e., 1vvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the cells were isolated and analyzed for C100 expression.

Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by Western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364 amino acids. By gel analysis the expressed polypeptide has a MW_r of 39.9K.

Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These results suggest that the expressed C100 polypeptide may be insoluble.

Example 3: Expression of HCV Antigen S2

HCV antigen S2 contains a sequence from the hydrophobic N-terminus of the S domain. It includes amino acids 199-328 of Figure 1.

The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described in Example 2.

The template for the PCR reaction was the vector pBR322/Pi14a, which had been linearized by digestion with HindIII. Pi14a is a cDNA clone that encodes amino acids 199-328.

The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following. For the 5'-region of the S2 sequence:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT
ACC ACG TCA CCA ATG ATT GCC CTA AC 3';

and

for the 3'-region of the S2 sequence:

5'GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC
ATC ATC ATA TCC CAT GCC AT 3'.

The primer for the 5'-region introduces a HindIII site and an ATG start codon into the amplified product. The primer for the 3'-region introduces translation stop codons and a SalI site into the amplified product.

The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from pBR322 digested with HindIII and SalI fragment, yielding the plasmid pBR322/S2d.

Ligation of the 413 bp HindIII-SalI S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.

Example 4: Synthesis of HCV C Antigen

HCV antigen C22 is from the C domain. It comprises amino acids 1-122 of Figure 1.

The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described supra, except for the following.

The template for the PCR reaction was pBR322/ Ag30a which had been linearized with HindIII. Ag30 is a cDNA clone that encodes amino acids 1-122. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

For the 5'-region of the C sequence:

5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA
ATC CTA AAC CTC AAA AAA AAA AC 3',

and
for the 3'-region of the C sequence:

5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC
GAC CTA CGC CGG GGG TCT GT 3'.

The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a SalI site. The PCR was run for 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the SalI-HindIII large SalI-HindIII fragment of pBR322 yielded the plasmid pBR322/C2.

Ligation of the 381 bp HindIII-SalI C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu$^-$ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW_r of approximately 13.6 Kd.

Example 5: Synthesis of NS5 Polypeptide

This polypeptide contains sequence from the N-terminus of the NS5 domain. Specifically it includes amino acids 2054 to 2464 of Figure 1. The protocol for the construction of the expression vector encoding the NS5 polypeptide and for its expression were analogous to that used for the expression of C33c (see Example 1).

Example 6: Radioimmunoassay (RIA) for Antibodies to HCV

The HCV antigens of Examples 1-5 were tested in an RIA format for their ability to detect antibodies to HCV in the serum of individuals clinically diagnosed as having HCV (Non-A, Non-B) and in serum from blood given by paid blood donors.

The RIA was based upon the procedure of Tsu and Herzenberg (1980) in SELECTED METHODS IN CELLULAR IMMUNOLOGY (W.H. Freeman & Co.), pp. 373-391. Generally, microtiter plates (Immulon 2, Removawell strips) are coated with purified HCV antigen. The coated plates are incubated with the serum samples or appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with ^{125}I-labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

Specifically, one hundred microliter aliquots containing 0.1 to 0.5 micrograms of the HCV antigen in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the antigen solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (TM) (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The antigens in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Antibody bound to the antigen was determined by the binding of ^{125}I-labeled $F'(ab)_2$ sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

Table 1 below presents the results of the tests on the serum from individuals diagnosed as having HCV.

Table 1

INDIVIDUAL	ANTIGEN				
	S2	C22	C100	C33c	NS5
CVH IVDA	P	P	P(+++)	P	P
CVH IVDA	P	P	P(+)	P	P
CVH IVDA	P	P	P(+)	P	P
CVH NOS	P	P	P	P	P
AVH NOS HS	N	N	N	N	N
AVH NOS HS	P	N	N	N	N
AVH NOS HS	P	N	N	N	N
AVH NOS HS	P/N	N	N	N	N
AVH PTVH	N	N	N	P/N	N
AVH NOS HS	N	N	N	N	N
AVH NOS	N	N	N	N	P
AVH PTVH	N	N	N	N	N
AVH IVDA	N	P	N	P	P
AVH PTVH	P	P/N	N	N	P
AVH NOS	N	P	N	N	N
AVH IVDA	N	P	N	P	P
AVH NOS HS	P/N	N	N	N	N
AVH PTVH	N	N	N	N	N
CVH IVDA	P	P	P	P	P
CVH IVDA	P	P	P	P	P
AVH NOS HS	N	N	N	N	N
CVH PTVH	P	P	N	N	N
AVH PTVH	P	N	P(+)	P(+++)	N
CVH PTVH	N	P	P	P	P
CVH NOS HS	P	P	P	P	N
CVH NOS	N	P	P/N	P	P

INDIVIDUAL	ANTIGEN				
	S2	C22	C100	C33c	NS5
CVH IVDA	N	N	N	P	N
AVH IVDA	P	P	P	P	P
AVH IVDA	P	P	P	P	P
CVH IVDA	P	P	P	P	P
AVH IVDA	P/N	P	N	P	P
AVH IVDA	N	P	P	P	N
CVH PTVH	P	P/N	N	N	N
CVH NOS	N	N	N	N	N
CVH NOS	N	N	N	N	N
CVH IVDA	P	P	P	P	P
AVH IVDA	P	P	P	P	P
CVH PTVH	P	P	P	P	P
AVH PTVH?	N	P	P	P	P
AVH IVDA	N	P	N	P	N
AVH NOS	N	N	N	N	N
AVH NOS	N	N	N	N	N
CVH NOS	N	P	N	N	P
CVH NOS	P	P	N	N	N
CVH NOS HS	P	P	P	P	P
CVH PTVH	P	P	N	P	P
AVH nurse	P	P	N	N	N
AVH IVDA	P	P	P	P	N
AVH IVDA	N	P	P(+)	P(+++)	N
AVH nurse	P/N	P	N	N	N
AVH PTVH	P/N	P	P	N	P
AVH NOS	N	P/N	N	N	P
AVH NOS	N	P	N	P	N
AVH PTVH	P	P/N	N	N	N
AVH PTVH	N	P	N	P	P
AVH PTVH	P	P	P	P	P
AVH PTVH	N	P	N	N	P
CVH PTVH	P/N	P	P(+)	P(+++)	N
AVH PTVH	N	P/N	P(+)	P(+++)	P

INDIVIDUAL		ANTIGEN				
	S2	C22	C100	C33c	NS5
AVH PTVH	P	(?)	P	N	P
CVH PTVH	N	P	N	P	P
CVH PTVH	N	P	P	P	P
CVH PTVH	N	N	N	N	N
AVH NOS	N	N	N	N	N
AVH nurse	P	P	N	N	N
CVH PTVH	N	P	N	N	P
AVH IVDA	N	P	N	P/N	N
CVH PTVH	P	P	P(+)	P(+++)	P
AVH NOS	P	P	N	N	N
AVH NOS	P/N	P	N	N	P
AVH PTVH	P/N	P	P	P	P
AVH NOS	N	P	P	P	P/N
AVH IVDA	N	P	N	N	P
AVH NOS	N	P/N	N	N	N
AVH NOS	P	P	N	N	P
AVH PTVH	N	P	P	P	P
crypto	P	P	P	P	P
CVH NOS	N	P	P	P	P
CVH NOS	N	N	N	N	N
AVH PTVH	N	P	P(+)	P(++)	N
AVH PTVH	N	P/N	P(+)	P(++)	P
AVH PTVH	N	P/N	P(+)	P(++)	P
CVH IVDA	P	P	P	P	P
CVH IVDA	P	P	P	P	P
CVH IVDA	P	P	P	P	P
CVH IVDA	P	P	P	P	P
AVH NOS	N	P	N	N	N
CVH IVDA	P	P	P	P	P/N
AVH IVDA	P	P	P	P	N
AVH NOS	P	P	N	N	N
AVH NOS	P	P	N	N	N
CVH PTVH	P	P	N	N	P/N

INDIVIDUAL		ANTIGEN				
	S2	C22	C100	C33c	NS5
AVH PTVH	N	P	N	P	P
AVH NOS	N	N	N	N	N
AVH NOS	N	P	N	N	N
AVH NOS	P	N	N	N	N
CVH NOS	N	N	N	N	N
AVH NOS	N	P/N	N	N	N
AVH IVDA	N	P	P	P	P
crypto	N	P	N	N	P/N
crypto	P	P	P/N	P	P
AVH IVDA	N	N	P	P	N
AVH IVDA	N	P	P	P	N
AVH NOS	N	N	N	N	N
AVH NOS	N	N	N	N	N
CVH IVDA	P	P	P	P	P
CVH PTVH	N	N	N	N	N
CVH PTVH	P	P	P(+)	P(+++)	P
CVH PTVH	P	P	P(+)	P(+++)	P
CVH NOS	P/N	N	N	N	N
CVH NOS	N	N	N	N	N
CVH PTVH	P	P	P	P	P
CVH PTVH	P	P	P	P	P
CVH PTVH	P	P	P	P	P
AVH IVDA	N	P	P	P	P
CVH NOS	N	N	N	N	N
CVH NOS	N	N	N	N	N
CVH PTVH	P	P	P	P	P
AVH NOS	P	P	N	N	P/N
AVH NOS	N	P/N	N	N	N
CVH PTVH	P	P	N	N	P
CVH NOS	N	P/N	N	N	N
AVH NOS	N	P	N	N	N
AVH NOS	N	P	N	N	N
CVH PTVH	N	P	N	N	N

INDIVIDUAL	ANTIGEN				
	S2	C22	C100	C33c	NS5
AVH IVDA	N	P	N	P	P
AVH NOS	P	N	N	N	N
CVH NOS	N	N	N	N	N
CVH NOS	N	N	N	N	N
CVH IVDA	P	P	P	P	P
CVH IVDA	P/N	P	P	P	P
CVH IVDA	P	P	P	P	P
CVH IVDA	N	P	P	P	P
AVH NOS	N	P	N	N	N
CVH IVDA	N	P	N	N	P
CVH IVDA	N	P	N	N	P
AVH PTVH	P	P	N	P	P
AVH PTVH	P	P	N	P	P
CVH NOS	N	P/N	N	N	P/N
CVH NOS	N	P	N	N	N
CVH NOS	N	N	N	N	N
CVH PTVH	P	P	P	P	P
CVH PTVH	P	P	P	P	P
CVH PTVH	P	P	P	P	P
AVH IVDA	N	P	N	N	P
AVH IVDA	N	P	P(++)	P(+)	P
CVH PTVH	P	P	P	P	P
AVH PTVH	N	P	P	P	P
CVH PTVH?	N	P	P	P	P
CVH PTVH?	P/N	P	P	P	P
CVH NOS HS	P	P	N	N	N
CVH IVDA	P	P	P	P	N
CVH PTVH	N	P	P	P	P
CVH PTVH	P	P	P	P	P/N
CVH NOS	P	P	P	P	P
CVH IVDA	P	P	P	P	P
CVH PTVH	P	P	P	P	N
CVH PTVH	P	P	P	P	P

INDIVIDUAL	ANTIGEN				
	S2	C22	C100	C33c	NS5
CVH NOS	N	N	N	N	P/N
CVH NOS	N	P/N	N	N	P/N
CVH PTVH	P	P	P	P	P
CVH NOS	N	P	N	N	N
CVH NOS	N	N	N	N	N
CVH NOS	P	P	N	N	P/N
CVH NOS	N	N	N	N	N
CVH NOS HS	P	P	P	P	P
CVH NOS HS	P	P	P	P	P
CVH PTVH	N	N	N	N	N
AVH PTVH	N	P	P	P	P
AVH NOS			-	-	
CVH PTVH	N	P	P/N	P(+++)	N
crypto	P	P	P	P	P
crypto	P	P	P	P	P
crypto	N	P	N	N	N
crypto	N	P	P	P	P
CVH PTVH	P	P	P	P	P
crypto	N	N	N	N	N
crypto	N	P	N	N	P/N
crypto	N	P	N	N	P
crypto	P	P	P	P	P
crypto	N	P	N	P	N
crypto			-	-	
crypto			-	-	
CVH NOS			-	-	
AVH-IVDA	N	P	N	P(+)	P

INDIVIDUAL	ANTIGEN				
	S2	C22	C100	C33c	NS5
AVH-IVDA	N	P/N	N	P(++)	N

AVH = acute viral hepatitis
CVH = chronic viral hepatitis
PTVH = post-transfusion viral hepatitis
IVDA = intravenous drug abuser
crypto = cryptogenic hepatitis
NOS = non-obvious source
P = positive
N = negative

Per these results, no single antigen reacted with all sera. C22 and C33c were the most reactive and S2 reacted with some sera from some putative acute HCV cases with which no other antigen reacted. Based on these results, the combination of two antigens that would provide the greatest range of detection is C22 and C33c. If one wished to detect a maximum of acute infections, S2 would be included in the combination.

Table 2 below presents the results of the testing on the paid blood donors.

Table 2

	Antigens				
Donor	C100	C33c	C22	S2	NS5
1	N	N	N	N	N
2	N	N	N	N	N
3	P	P	P	P	P
4	N	N	N	N	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N

	Antigens				
Donor	C100	C33c	C22	S2	NS5
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N
15	N	N	N	N	N
16	N	N	N	N	N
17	N	N	N	N	N
18	P	P	P	P	P
19	P	P	N	P	P
20	P	P	N	P	P
21	N	N	N	N	N
22	N	P	P	N	P
23	P	P	P	P	P
24	N	N	N	N	N
25	N	N	N	N	N
26	N	N	N	N	N
27	N	N	N	N	N
28	N	N	N	N	N
29	N	N	N	N	N
30	N	N	N	N	N
31	P	P	P	N	P
32	N	N	N	N	N
33	N	N	N	N	N
34	N	N	N	N	P
35	N	N	P	N	P
36	N	N	N	N	N
37	N	N	N	N	N
38	N	N	N	N	N
39	N	N	N	N	N
40	N	N	N	N	N
41	N	N	N	N	P
42	N	N	N	N	N

	Antigens				
Donor	C100	C33c	C22	S2	NS5
43	N	N	N	N	N
44	N	N	N	N	N
45	N	N	N	N	N
46	N	N	N	N	N
47	P	P	N	N	P
48	N	N	N	N	N
49	N	N	N	N	N
50	N	N	N	N	N
51	N	P	P	N	P
52	N	N	N	N	N
53	N	P	P	N	P
54	P	P	P	P	N
55	N	N	N	N	N
56	N	N	N	N	N
57	N	N	N	N	N
58	N	N	N	N	N
59	N	N	N	N	N
60	N	N	N	N	N
61	N	N	N	N	N
62	N	N	N	N	N
63	N	N	N	N	N
64	N	N	N	N	N
65	N	N	N	N	N
66	N	N	N	N	N
67	N	N	N	N	N
68	N	N	N	N	N
69	N	N	N	N	N
70	P	P	P	P	P
71	N	N	N	N	N
72	N	N	N	N	N
73	P	P	P	P	N
74	N	N	N	N	N
75	N	N	N	N	N
76	N	N	N	N	P

	Antigens				
Donor	C100	C33c	C22	S2	NS5
77	N	N	N	N	N
78	N	N	N	N	N
79	N	N	N	N	N
80	N	N	N	N	N
81	N	N	N	N	N
82	N	N	N	N	N
83	P	P	N	N	N
84	N	N	P	N	N
85	N	N	N	N	N
86	P	P	P	P	N
87	N	N	N	N	N
88	N	N	N	N	N
89	P	P	P	P	P
90	P	P	P	P	N
91	N	N	N	N	P
92	P	P	P	N	N
93	N	N	N	N	N
94	N	N	N	N	N
95	N	N	N	N	N
96	N	N	N	N	N
97	N	N	N	N	N
98	N	P	P	P	P
99	P	P	P	P	P
100	N	N	N	N	N
101	P	P	P	P	P
102	N	N	N	N	N
103	N	N	N	N	N
104		N	N	N	N
105	P	P	P	P	N
106	N	N	N	N	N
107	N	N	N	N	N
108	N	N	N	N	N
109	P	P	P	P	P
110	P	P	P	N	P

	Antigens				
Donor	C100	C33c	C22	S2	NS5
111	P	P	P	N	P
112	N	N	N	N	N
113	P	P	P	P	P
114	N	N	N	N	N
115	N	N	N	N	N
116	P	P	P	P	P
117	N	N	N	N	N
118	N	N	N	N	N
119	N	N	N	N	N
120	P	P	P	P	P
121	N	N	N	N	N
122	N	P	P	N	P
123	N	N	N	N	N
124	N	N	N	N	N
125	N	N	N	N	N
126	P	N	N	N	N
127	N	N	N	N	N
128	N	N	N	N	N
129	N	N	N	N	N
130	P	P	P	P	N
131	N	N	N	N	P
132	N	N	N	N	N
133	N	N	N	N	N
134	N	N	N	N	N
135	N	N	N	N	N
136	N	N	N	N	N
137	N	N	N	N	N
138	N	N	N	N	N
139	N	N	N	N	N
140	P	N	N	N	N
141	P	N	P	P	P
142	N	N	N	N	N
143	N	N	N	N	N
144	N	N	N	N	N

	Antigens				
Donor	C100	C33c	C22	S2	NS5
145	N	N	N	N	N
146	N	N	N	N	N
147	N	N	N	N	N
148	N	N	N	N	N
149	N	N	N	N	N
150	N	N	N	N	N
151	N	N	N	N	N
152	N	N	N	N	N
153	N	N	N	N	N
154	P	P	P	P	P
155	N	N	N	N	N
156	N	N	N	N	N
157	N	N	N	N	N
158	N	N	N	N	N
159	N	N	N	N	N
160	N	N	N	N	N
161	P	P	P	P	P
162	N	N	N	N	N
163	N	N	N	N	N
164	P	P	P	N	P
165	N	N	N	N	N
166	P	P	P	N	P
167	N	N	N	N	N
168	N	N	N	N	N
169	N	N	N	N	N
170	N	N	N	N	N
171	N	N	N	N	N
172	N	N	N	N	N
173	N	N	N	N	N
174	N	N	N	N	N
175	N	N	N	N	N
176	N	N	N	N	N
177	N	N	N	N	P
178	N	N	N	N	N

	Antigens				
Donor	C100	C33c	C22	S2	NS5
179	N	N	N	N	N
180	N	N	N	N	N
181	N	N	N	N	N
182	N	N	N	N	N
183	P	P	P	P	P
184	N	N	N	N	N
185	N	N	N	N	N
186	N	N	N	N	N
187	N	N	N	N	N
188	N	P	P	N	N
189	N	N	N	N	N
190	N	N	N	N	N
191	N	N	N	N	N
192	N	N	N	N	N
193	N	N	N	N	N
194	N	N	N	N	N
195	N	N	N	N	N
196	N	N	N	N	N
197	N	N	N	N	P
198	P	P	P	N	N
199	N	N	N	N	P
200	P	P	P	P	N

The results on the paid donors generally confirms the results from the sera of infected individuals.

Example 7: ELISA Determinations of HCV Antibodies Using Combination of HCV Antigens

Plates coated with a combination of C22 and C33c antigens are prepared as follows. A solution containing coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA (25 micrograms/ml), C22 and C33c (2.50 micrograms/ml each) is prepared just prior to addition to the Removeawell Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution is added to the plates, they are covered and incubated for 2 hours at 37°C, after which the solution is removed by aspiration. The wells are washed once with 400 microliters wash buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100(TM), 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethylsulfonylfluoride (PMSF)) is added, the plates are loosely covered to prevent evaporation, and are allowed to stand at room temperature for 30 minutes. The wells are then aspirated to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches with dessicant (3 g Sorb-it™ packs).

In order to perform the ELISA determination, 20 microliters of serum sample or control sample is added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100(TM), 100 micrograms/ml yeast extract). The plates are sealed, and are incubated at 37°C for two hours, after which the solution is removed by aspiration, and the wells are washed three times with 400 microliters of wash buffer -(phosphate buffered saline (PBS) containing 0.05% Tween 20(TM). The washed wells are treated with 200 microliters of mouse anti-human IgG-horse radish peroxidase (HRP) conjugate contained in a solution of Ortho conjugate diluent (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50% (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM $K_3Fe(CN)_6$, 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment is for 1 hour at 37°C, the solution is removed by aspiration, and

the wells are washed three times with 400 ml wash buffer, which is also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) is added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H_2O_2. The plates containing the substrate solution are incubated in the dark for 30 minutes at room temperature, the reactions are stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

In a similar manner, ELISAs using fusion proteins of C22 and C33c, and C22, C33c, and S2 and combinations of C22 and C100, C22 and S2, C22 and an NS5 antigen, C22, C33c, and S2, and C22, C100, and S2 may be carried out.

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GR, IT, LU, NL, SE

1. A combination of hepatitis C virus (HCV) epitope sequences in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

   (a) a first epitope sequence from the C domain of the HCV polyprotein;
   (b) a second epitope sequence from a second domain of the HCV polyprotein which domain is:

      (i) the NS3 domain of the HCV polyprotein;
      (ii) the NS4 domain of the HCV polyprotein; or
      (iii) the NS5 domain of the HCV polyprotein; and

   (c) a third epitope sequence from a third domain of the HCV polyprotein which domain is:

      (i) the NS3 domain of the HCV polyprotein;
      (ii) the NS4 domain of the HCV polyprotein; or
      (iii) the NS5 domain of the HCV polyprotein;

   wherein the third domain is different from the second domain;
   with the proviso that the combination is not the peptide p1 with C100-3, the peptide p35 with C100-3 or the peptide p99 with C100-3.

2. A combination according to claim 1 wherein the second domain is NS3.

3. A combination according to claim 1 wherein the second domain is NS4.

4. A combination according to claim 1 which comprises:

   (a) a first epitope sequence from the C domain of the HCV polyprotein;
   (b) a second epitope sequence from the NS3 domain of the HCV polyprotein; and
   (c) a third epitope sequence from the NS4 domain of the HCV polyprotein.

5. A combination according to any one of claims 1 to 4 wherein the solid matrix is the surface of a microtiter plate well, a bead or dipstick.

6. A combination according to any one of claims 1 to 5 wherein the first, second and third epitope sequences are contained in first, second and third polypeptides respectively individually bound to the solid matrix.

7. A combination according to claim 6 wherein the solid matrix is a dipstick and the polypeptides are distributed individually in a pattern such that binding to the first, second and third polypeptides may be discerned.

8. The combination of any one of claims 1 to 5 wherein the combination is in the form of a fusion polypeptide.

9. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of con-

taining said antibodies comprising contacting said body component with the combination of any one of claims 1 to 8 under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said polypeptide epitope sequences.

10. A kit for carrying out an assay for detecting antibodies to hepatitis C antigen (HCV) in a mammalian body component suspected of containing said antibodies comprising in packaged combination:

(a) the combination of HCV epitope sequences of any one of claims 1 to 8,
(b) standard control reagents; and
(c) instructions for carrying out the assay.

Claims for the following Contracting State : ES

1. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the combination of polypeptide HCV epitope sequences under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said polypeptide epitope sequences, wherein epitope sequences are in one or more polypeptides made by chemical synthesis or recombinant expression, immobilized on the surface of a solid matrix suitable for detection of HCV by immunoassay, comprising:

(a) a first epitope sequence from the C domain of the HCV polyprotein;
(b) a second epitope sequence from a second domain of the HCV polyprotein which domain is:

(i) the NS3 domain of the HCV polyprotein;
(ii) the NS4 domain of the HCV polyprotein; or
(iii) the NS5 domain of the HCV polyprotein; and

(c) a third epitope sequence from a third domain of the HCV polyprotein which domain is:

(i) the NS3 domain of the HCV polyprotein;
(ii) the NS4 domain of the HCV polyprotein; or
(iii) the NS5 domain of the HCV polyprotein;

wherein the third domain is different from the second domain;
with the proviso that the combination is not the peptide p1 with C100-3, the peptide p35 with C100-3 or the peptide p99 with C100-3.

2. A method according to claim 1 wherein the second domain is NS3.

3. A method according to claim 1 wherein the second domain is NS4.

4. A method according to claim 1 in which the combination comprises:

(a) a first epitope sequence from the C domain of the HCV polyprotein;
(b) a second epitope sequence from the NS3 domain of the HCV polyprotein; and
(c) a third epitope sequence from the NS4 domain of the HCV polyprotein.

5. A method according to any one of claims 1 to 4 wherein the solid matrix is the surface of a microtiter plate well, a bead or dipstick.

6. A method according to any one of claims 1 to 5 wherein the first, second and third epitope sequences are contained in first, second and third polypeptides respectively individually bound to the solid matrix.

7. A method according to claim 6 wherein the solid matrix is a dipstick and the polypeptides are distributed individually in a pattern such that binding to the first, second and third polypeptides may be discerned.

8. The method of any one of claims 1 to 5 wherein the combination is in the form of a fusion polypeptide.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GR, IT, LI, LU, NL, SE

1. Kombination von Hepatitis-C-Virus-(HCV-)Epitopsequenzen in einem oder mehreren Polypeptid(en), hergestellt durch chemische Synthese oder rekombinante Expression, immobilisiert auf der Oberfläche einer festen Matrix, mit der Eignung zum Nachweis von HCV in einem Immunoassay, umfassend:

   (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polyproteins;
   (b) eine zweite Epitopsequenz aus einer zweiten Domäne des HCV-Polyproteins, wobei die Domäne:

      (i) die NS3-Domäne des HCV-Polyproteins;
      (ii) die NS4-Domäne des HCV-Polyproteins; oder
      (iii) die NS5-Domäne des HCV-Polyproteins ist; und

   (c) eine dritte Epitopsequenz aus einer dritten Domäne des HCV-Polyproteins, wobei die Domäne:

      (i) die NS3-Domäne des HCV-Polyproteins;
      (ii) die NS4-Domäne des HCV-Polyproteins; oder
      (iii) die NS5-Domäne des HCV-Polyproteins ist;

   wobei die dritte Domäne sich von der zweiten Domäne unterscheidet;
   mit der Maßgabe, daß die Kombination nicht das Peptid pI mit C100-3, das Peptid p35 mit C100-3 oder das Peptid p99 mit C100-3 ist.

2. Kombination nach Anspruch 1, worin die zweite Domäne NS3 ist.

3. Kombination nach Anspruch 1, worin die zweite Domäne NS4 ist.

4. Kombination nach Anspruch 1, umfassend:

   (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polyproteins;
   (b) eine zweite Epitopsequenz aus der NS3-Domäne des HCV-Polyproteins; und
   (c) eine dritte Epitopsequenz aus der NS4-Domäne des HCV-Polyproteins.

5. Kombination nach einem der Ansprüche 1 bis 4, worin die feste Matrix die Oberfläche einer Kavität einer Mikrotiterplatte, eines Kügelchens oder eines Tauchstäbchens ist.

6. Kombination nach einem der Ansprüche 1 bis 5, worin die ersten, zweiten und dritten Epitopsequenzen in den ersten, zweiten bzw. dritten Polypeptiden in individueller Bindung an die feste Matrix enthalten sind.

7. Kombination nach Anspruch 6, worin die feste Matrix ein Tauchstäbchen ist und die Polypeptide individuell in einem Muster so verteilt sind, daß die Bindung an das erste, zweite und dritte Polypeptid unterschieden werden kann.

8. Kombination nach einem der Ansprüche 1 bis 5, worin die Kombination in Form eines Fusions-Polypeptids vorliegt.

9. Verfahren zum Nachweis von Antikörpern gegen das Hepatitis-C-Virus (HCV) in einer Körperkomponente eines Säugers, der vermutlich die Antikörper enthält, wobei man die Körperkomponente mit der Kombination nach einem der Ansprüche 1 bis 8 unter Bedingungen in Kontakt bringt, die eine Antikörper-Antigen-Reaktion erlauben, und die Anwesenheit von Immunkomplexen aus den Antikörpern und den Polypeptid-Epitopsequenzen nachweist.

10. Kit zur Durchführung eines Assays zum Nachweis von Antikörpern gegen das Hepatitis-C-Antigen (HCVI in einer Körperkomponente eines Säugers, der vermutlich die Antikörper enthält, umfassend in abgepackter Kombination:

    (a) die Kombination aus HCV-Epitopsequenzen nach einem der Ansprüche 1 bis 9;
    (b) Standard-Kontrollreagentien; und
    (c) Anweisungen zur Durchführung des Assays.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zum Nachweis von Antikörpern gegen das Hepatitis-C-virus (HCV) in einer Körperkomponente eines Säugers, der vermutlich die Antikörper enthält, wobei man die Körperkomponente mit der Kombination von Polypeptid-HCV-Epitopsequenzen unter Bedingungen in Kontakt bringt, die eine Antikörper-Antigen-Reaktion erlauben, und die Anwesenheit von Immunkomplexen aus den Antikörpern und den Polypeptid-Epitopsequenzen nachweist, wobei die Epitopsequenzen in einem oder mehreren Polypeptid(en) vorhanden sind, die durch chemische Synthese oder rekombinante Expression hergestellt wurden, auf der Oberfläche einer festen Matrix immobilisiert sind und zum Nachweis von HCV durch einen Immunoassay geeignet sind, umfassend:

   (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polyproteins;
   (b) eine zweite Epitopsequenz aus einer zweiten Domäne des HCV-Polyproteins, wobei die Domäne:

      (i) die NS3-Domäne des HCV-Polyproteins;
      (ii) die NS4-Domäne des HCV-Polyproteins; oder
      (iii) die NS5-Domäne des HCV-Polyproteins ist; und

   (c) eine dritte Epitopsequenz aus einer dritten Domäne des HCV-Polyproteins, wobei die Domäne:

      (i) die NS3-Domäne des HCV-Polyproteins;
      (ii) die NS4-Domäne des HCV-Polyproteins; oder
      (iii) die NS5-Domäne des HCV-Polyproteins ist;

   wobei die dritte Domäne sich von der zweiten Domäne unterscheidet;
   mit der Maßgabe, daß die Kombination nicht das Peptid pI mit C100-3, das Peptid p35 mit C100-3 oder das Peptid p99 mit C100-3 ist.

2. Verfahren nach Anspruch 1, wobei die zweite Domäne NS3 ist.

3. Verfahren nach Anspruch 1, wobei die zweite Domäne NS4 ist.

4. Verfahren nach Anspruch 1, wobei die Kombination umfaßt:

   (a) eine erste Epitopsequenz aus der C-Domäne des HCV-Polyproteins;
   (b) eine zweite Epitopsequenz aus der NS3-Domäne des HCV-Polyproteins; und
   (c) eine dritte Epitopsequenz aus der NS4-Domäne des HCV-Polyproteins.

5. Verfahren nach einem der Ansprüche 1 bis 4, wobei die feste Matrix die Oberfläche einer Kavität einer Mikrotiterplatte, eines Kügelchens oder eines Tauchstäbchens ist.

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei die ersten, zweiten und dritten Epitopsequenzen in den ersten, zweiten bzw. dritten Polypeptiden in individueller Bindung an die feste Matrix enthalten sind.

7. Verfahren nach Anspruch 6, wobei die feste Matrix ein Tauchstäbchen ist und die Polypeptide individuell in einem Muster so verteilt sind, daß die Bindung an das erste, zweite und dritte Polypeptid unterschieden werden kann.

8. Verfahren nach einem der Ansprüche 1 bis 5, wobei die Kombination in Form eines Fusions-Polypeptids vorliegt.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GR, IT, LI, LU, NL, SE

1. Combinaison de séquences épitopiques de virus de l'hépatite C (HCV) dans un ou plusieurs polypeptides produits par synthèse chimique ou par expression recombinante, immobilisée à la surface d'une matrice solide appropriée pour la détection du HCV par test immunologique, comprenant :

(a) une première séquence épitopique du domaine C de la polyprotéine de HCV ;

(b) une deuxième séquence épitopique d'un deuxième domaine de la polyprotéine de HCV, domaine qui est :

(i) le domaine NS3 de la polyprotéine de HCV ;
(ii) le domaine NS4 de la polyprotéine de HCV ; ou
(iii) le domaine NS5 de la polyprotéine de HCV ; et

(c) une troisième séquence épitopique d'un troisième domaine de la polyprotéine de HCV, domaine qui est :

(i) le domaine NS3 de la polyprotéine de HCV ;
(ii) le domaine NS4 de la polyprotéine de HCV ; ou
(iii) le domaine NS5 de la polyprotéine de HCV ;

le troisième domaine étant différent du deuxième domaine ;
avec la condition que la combinaison ne soit pas le peptide p1 avec C100-3, le peptide p35 avec C100-3 ou le peptide p99 avec C100-3.

2. Combinaison selon la revendication 1, dans laquelle le deuxième domaine est NS3.

3. Combinaison selon la revendication 1, dans laquelle le deuxième domaine est NS4.

4. Combinaison selon la revendication 1, qui comprend :

(a) une première séquence épitopique du domaine C de la polyprotéine de HCV;

(b) une deuxième séquence épitopique du domaine NS3 de la polyprotéine de HCV ; et

(c) une troisième séquence épitopique du domaine NS4 de la polyprotéine de HCV.

5. Combinaison selon l'une quelconque des revendications 1 à 4, dans laquelle la matrice solide est la surface d'un puits de plaque de microtitration, d'une bille ou d'une bandelette réactive.

6. Combinaison selon l'une quelconque des revendications 1 à 5, dans laquelle les première, deuxième et troisième séquences épitopiques sont contenues dans les premier, deuxième et troisième polypeptides respectivement, liés individuellement à la matrice solide.

7. Combinaison selon la revendication 6, dans laquelle la matrice solide est une bandelette réactive et les polypeptides sont distribués individuellement dans une disposition telle que les liaisons avec les premier, deuxième et troisième polypeptides puissent être distinguées.

8. Combinaison selon l'une quelconque des revendications 1 à 5, dans laquelle la combinaison est sous la forme d'un polypeptide de fusion.

9. Méthode pour détecter des anticorps dirigés contre le virus de l'hépatite C (HCV) dans un constituant corporel d'un mammifère dont on soupçonne qu'il contient lesdits anticorps, comprenant les étapes consistant à mettre en contact ledit constituant corporel avec la combinaison de l'une quelconque des revendications 1 à 8 dans des conditions qui permettent une réaction antigène-anticorps et à détecter la présence de complexes immuns desdits anticorps et desdites séquences polypeptidiques épitopiques.

10. Kit pour effectuer un test de détection d'anticorps dirigés contre un antigène de l'hépatite C (HCV) dans un constituant corporel d'un mammifère dont on soupçonne qu'il contient lesdits anticorps, comprenant dans un ensemble conditionné :

(a) la combinaison de séquences épitopiques de HCV de l'une quelconque des revendications 1 à 8;

b) des réactifs témoins étalons ; et

(c) des instructions pour effectuer le test.

Revendications pour l'Etat contractant suivant : ES

1. Méthode pour détecter des anticorps du virus de l'hépatite C (HCV) dans un constituant corporel d'un mammifère dont on soupçonne qu'il contient lesdits anticorps, comprenant les étapes consistant à mettre en contact ledit constituant corporel avec la combinaison de séquences polypeptidiques épitopiques de HCV, dans des conditions qui permettent une réaction antigène-anticorps, et à détecter la présence de complexes immuns desdits anticorps et desdites séquences polypeptidiques épitopiques, dans laquelle les séquences épitopiques sont dans un ou plusieurs polypeptides produits par synthèse chimique ou expression recombinante, immobilisés à la surface d'une matrice solide appropriée pour la détection de HCV par un test immunologique, comprenant :

   (a) une première séquence épitopique du domaine C de la polyprotéine de HCV ;

   (b) une deuxième séquence épitopique d'un deuxième domaine de la polyprotéine de HCV, domaine qui est :

      (i) le domaine NS3 de la polyprotéine de HCV;
      (ii) le domaine NS4 de la polyprotéine de HCV; ou
      (iii) le domaine NS5 de la polyprotéine de HCV ; et

   (c) une troisième séquence épitopique d'un troisième domaine de la polyprotéine de HCV, domaine qui est:

      (i) le domaine NS3 de la polyprotéine de HCV;
      (ii) le domaine NS4 de la polyprotéine de HCV ; ou
      (iii) le domaine NS5 de la polyprotéine de HCV ;

   le troisième domaine étant différent du deuxième domaine ;
   avec la condition que la combinaison ne soit pas le peptide p1 avec C100-3, le peptide p35 avec C100-3 ou le peptide p99 avec C100-3.

2. Méthode selon la revendication 1, dans laquelle le deuxième domaine est NS3.

3. Méthode selon la revendication 1, dans laquelle le deuxième domaine est NS4.

4. Méthode selon la revendication 1, dans laquelle la combinaison comprend :

   (a) une première séquence épitopique du domaine C de la polyprotéine de HCV;

   (b) une deuxième séquence épitopique du domaine NS3 de la polyprotéine de HCV ; et

   (c) une troisième séquence épitopique du domaine NS4 de la polyprotéine de HCV.

5. Méthode selon l'une quelconque des revendications 1 à 4, dans laquelle la matrice solide est la surface d'un puits de plaque de microtitration, d'une bille ou d'une bandelette réactive.

6. Méthode selon l'une quelconque des revendications 1 à 5, dans laquelle les première, deuxième et troisième séquences d'épitope sont contenues dans les premier, deuxième et troisième polypeptides respectivement, liés individuellement à la matrice solide.

7. Méthode selon la revendication 6, dans laquelle la matrice solide est une bandelette réactive et les polypeptides sont distribués individuellement dans une disposition telle que les liaisons aux premier, deuxième et troisième polypeptides puissent être distinguées.

8. Méthode selon l'une quelconque des revendications 1 à 5, dans laquelle la combinaison est sous la forme d'un polypeptide de fusion.

FIG. 1A

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-341  GCCAGCCCCCTGATGGGGGCGA
      CGGTCGGGGGACTACCCCCGCT

-319  CACTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAG
      GTGAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAGTGCGTCTTTCGCAGATC

-259  CCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATA
      GGTACCGCAATCATACTCACAGCACGTCGGAGGTCCTGGGGGGGAGGGCCCTCTCGGTAT

-199  GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGA
      CACCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCCTGCTGGCCCAGGAAAGAACCT

-139  TCAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGT
      AGTTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGGCGTTCTGACGATCGGCTCATCA

- 79  GTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAG
      CAACCCAGCGCTTTCCGGAACACCATGACGGACTATCCCACGAACGCTCACGGGGCCCTC

- 19  GTCTCGTAGACCGTGCACC
      CAGAGCATCTGGCACGTGG
                                  Arg   Thr
      MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln
   1  ATGAGCACGAATCCTAAACCTCAAAAAAAAAACAAACGTAACACCAACCGTCGCCCACAG
      TACTCGTGCTTAGGATTTGGAGTTTTTTTTTTGTTTGCATTGTGGTTGGCAGCGGGTGTC

      AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg
  61  GACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGG
      CTGCAGTTCAAGGGCCCACCGCCAGTCTAGCAACCACCTCAAATGAACAACGGCGCGTCC
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    GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
121 GGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGAGCGGTCGCAACCTCGAGGT
    CCGGGATCTAACCCACACGCGCGCTGCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA

    ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
181 AGACGTCAGCCTATCCCCAAGGCTCGTCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
    TCTGCAGTCGGATAGGGGTTCCGAGCAGCCGGGCTCCCGTCCTGGACCCGAGTCGGGCCC

    TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
241 TACCCTTGGCCCCTCTATGGCAATGAGGGCTGCGGGTGGGCGGGATGGCTCCTGTCTCCC
    ATGGGAACCGGGGAGATACCGTTACTCCCGACGCCCACCCGCCCTACCGAGGACAGAGGG

    ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly
301 CGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCCGGCGTAGGTCGCGCAATTTGGGT
    GCACCGAGAGCCGGATCGACCCCGGGGTGTCTGGGGGCCGCATCCAGCGCGTTAAACCCA

    LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
361 AAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTC
    TTCCAGTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG

    GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
421 GGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGAC
    CCGCGGGGAGAACCTCCGCGACGGTCCCGGGACCGCGTACCGCAGGCCCAAGACCTTCTG
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FIG. 1B

FIG. 1C

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                                                                Thr
    GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
481 GGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTATCTTCCTTCTGGCC
    CCGCACTTGATACGTTGTCCCTTGGAAGGACCAACGAGAAAGAGATAGAAGGAAGACCGG

    LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
541 CTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGTGCGCAACTCCACGGGGCTT
    GACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTCACGCGTTGAGGTGCCCCGAA

    TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
601 TACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGCCGATGCCATC
    ATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCGCCGGCTACGGTAG

    LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
661 CTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAACGCCTCGAGGTGTTGGGTG
    GACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACAACCCAC

    AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
721 GCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCCCGCGACGCAGCTTCGACGT
    CGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGCTGCGTCGAAGCTGCA

    HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
781 CACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGCCCTCTACGTGGGGGACCTA
    GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGCACCCCCTGGAT

    CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
841 TGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTCTCCCAGGCGCCACTGGACG
    ACGCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAGAGGGTCCGCGGTGACCTGC
```

```
      ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
901   ACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACGGGTCACCGCATGGCATGG
      TGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCCAGTGGCGTACCGTACC

                                                        Val
      AspMetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle
961   GATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATC
      CTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCTAG

      ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
1021  CCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTGGCGGGCATAGCG
      GGTGTTCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCGTATCGC

      TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuLeuPheAlaGly
1081  TATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGTGCTGCTGCTATTTGCCGGC
      ATAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCG

      ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
1141  GTCGACGCGGAAACCCACGTCACCGGGGGAAGTGCCGGCCACACTGTGTCTGGATTTGTT
      CAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGTGTGACACAGACCTAAACAA

      SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
1201  AGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGATCAACACCAACGGCAGTTGG
      TCGGAGGAGCGTGGTCCGCGGTTCGTCTTGCAGGTCGACTAGTTGTGGTTGCCGTCAACC
```

FIG. 1D

FIG. 1E

```
        HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly
1261    CACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAACACCGGCTGGTTGGCAGGG
        GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC

        LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
1321    CTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGAGAGGCTAGCCAGCTGCCGA
        GAAAAGATAGTGGTGTTCAAGTTGAGAAGTCCGACAGGACTCTCCGATCGGTCGACGGCT

        ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
1381    CCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGGCCCC
        GGGGAATGGCTAAAACTGGTCCCGACCCCGGGATAGTCAATACGGTTGCCTTCGCCGGGG

        AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
1441    GACCAGCGCCCCTACTGCTGGCACTACCCCCCAAAACCTTGCGGTATTGTGCCCGCGAAG
        CTGGTCGCGGGGATGACGACCGTGATGGGGGGTTTTGGAACGCCATAACACGGGCGCTTC

        SerValCysGlyProValTyrCysPheThrProSerProValValValGlyThrThrAsp
1501    AGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGTGGTGGTGGGAACGACCGAC
        TCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCACCACCACCCTTGCTGGCTG

        ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn
1561    AGGTCGGGCGCGCCCACCTACAGCTGGGGTGAAAATGATACGGACGTCTTCGTCCTTAAC
        TCCAGCCCGCGCGGGTGGATGTCGACCCCACTTTTACTATGCCTGCAGAAGCAGGAATTG

        AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe
1621    AATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTGGATGAACTCAACTGGATTC
        TTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG
```

```
      ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
1681  ACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGGGGCGGGCAACAACACCCTGCAC
      TGGTTTCACACGCCTCGCGGAGGAACACAGTAGCCTCCCCGCCCGTTGTTGTGGGACGTG

      CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
1741  TGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATACTCTCGGTGCGGCTCCGGT
      ACGGGGTGACTAACGAAGGCGTTCGTAGGCCTGCGGTGTATGAGAGCCACGCCGAGGCCA

            Ile
      ProTrpLeuThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
1801  CCCTGGATCACACCCAGGTGCCTGGTCGACTACCCGTATAGGCTTTGGCATTATCCTTGT
      GGGACCTAGTGTGGGTCCACGGACCAGCTGATGGGCATATCCGAAACCGTAATAGGAACA

      ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
1861  ACCATCAACTACACCATATTTAAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGCTG
      TGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCCTCCCCAGCTTGTGTCCGAC

      GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
1921  GAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCC
      CTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGG

      GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr
1981  GAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTCCTCCCGTGTTCCTTCACA
      CTCGAGTCGGGCAATGACGACTGGTGATGTGTCACCGTCCAGGAGGGCACAAGGAAGTGT
```

FIG. 1F

FIG. 1G

```
     ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
2041 ACCCTACCAGCCTTGTCCACCGGCCTCATCCACCTCCACCAGAACATTGTGGACGTGCAG
     TGGGATGGTCGGAACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTC

     TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
2101 TACTTGTACGGGGTGGGGTCAAGCATCGCGTCCTGGGCCATTAAGTGGGAGTACGTCGTT
     ATGAACATGCCCCACCCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAA

     LeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
2161 CTCCTGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTC
     GAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAG

     IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
2221 ATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCC
     TATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCGTAGGGACCGG

     GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly
2281 GGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTTTGCATGGTATTTGAAGGGT
     CCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTACCATAAACTTCCCA

     LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuLeu
2341 AAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTGCTCCTG
     TTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACCGGAGAGGAGGACGAGGAC

     LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
2401 TTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGTGGCCGCGTCGTGTGGCGGT
     AACCGCAACGGGGTCGCCCGCATGCGCGACCTGTGCCTCCACCGGCGCAGCACACCGCCA
```

```
     ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
2461 GTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTATATCAGC
     CAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTATAATGTTCGCGATATAGTCG

                          (Asn)
     TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
2521 TGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAAGCGCAACTGCACGTGTGG
     ACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTGCACACC

     IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal
2581 ATTCCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCATCTTACTCATGTGTGCTGTA
     TAAGGGGGGGAGTTGCAGGCTCCCCCCGCGCTGCGGCAGTAGAATGAGTACACACGACAT

     HisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrp
2641 CACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCCGTCTTCGGACCCCTTTGG
     GTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGCAGAAGCCTGGGGAAACC

     IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
2701 ATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCGCGTCCAAGGCCTTCTCCGG
     TAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGCGCAGGTTCCGGAAGAGGCC

     PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
2761 TTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAATGGTCATCATTAAG
     AAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCACGTTTACCAGTAGTAATTC
```

FIG. 1H

FIG. 1I

```
     LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla
2821 TTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGACTGGGCG
     AATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCGC

     HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu
2881 CACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAAATGGAG
     GTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAGCAGAAGAGGGTTTACCTC

     ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu
2941 ACCAAGCTCATCACGTGGGGGGCAGATACCGCCGCGTGCGGTGACATCATCAACGGCTTG
     TGGTTCGAGTAGTGCACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAAC

     ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer
3001 CCTGTTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC
     GGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGGTCGGCTACCTTACCAGAGG

     LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu
3061 AAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTA
     TTCCCCACCTCCAACGACCGCGGGTAGTGCCGCATGCGGGTCGTCTGTTCCCCGGAGGAT

     GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln
3121 GGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAG
     CCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTTTTGGTTCACCTCCCACTCCAGGTC

     IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr
3181 ATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACT
     TAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCACACGACCTGA
```


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```
      ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
3241  GTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATG
      CAGATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTTCCCAGGACAGTAGGTCTAC

                                                  Ser       Thr
      TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu
3301  TATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTG
      ATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAAC

      ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
3361  ACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATT
      TGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTGCTCCGTGCGGCTACAGTAA

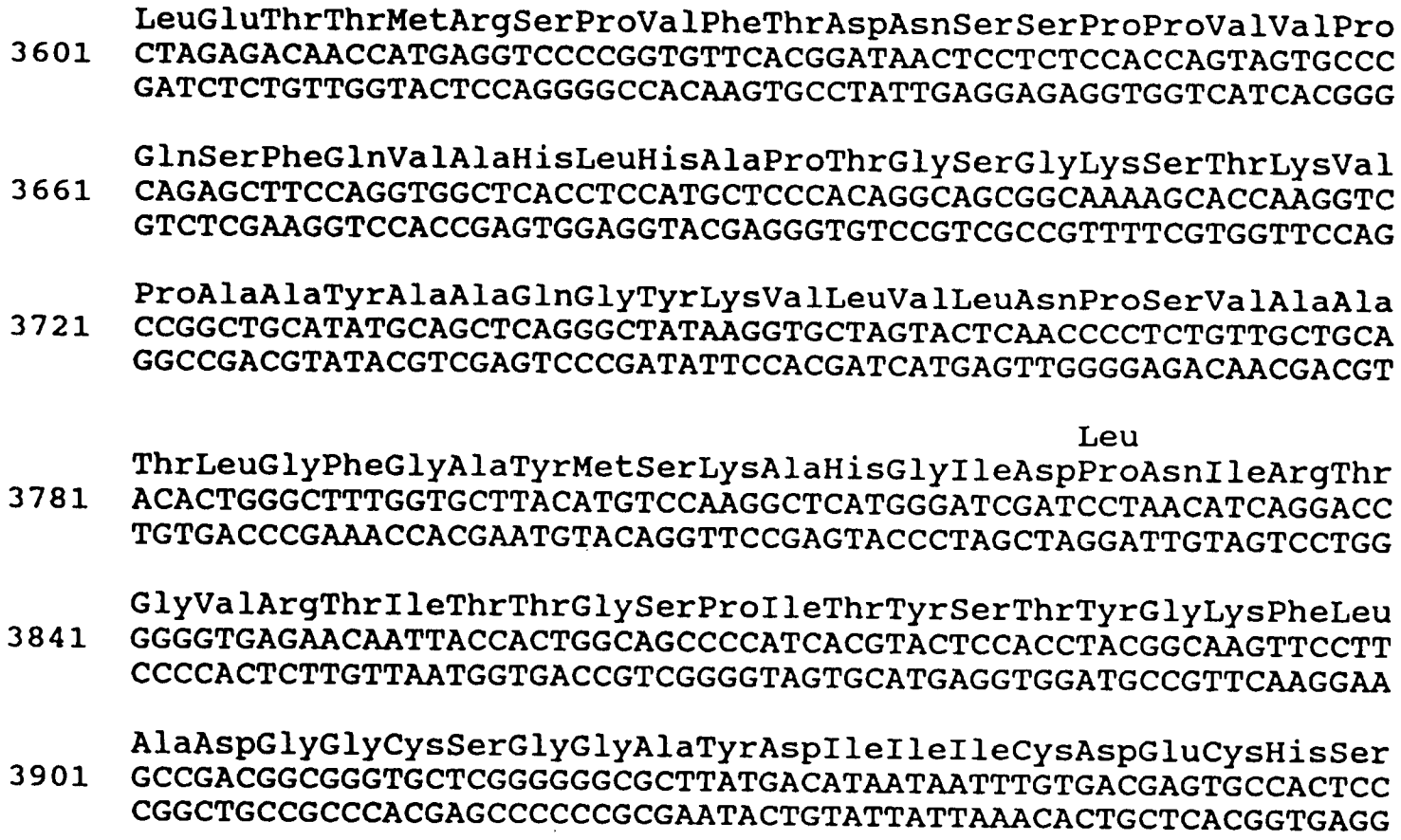
      ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
3421  CCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTAC
      GGGCACGCGGCCGCCCCACTATCGTCCCCGTCGGACGACAGCGGGGCCGGGTAAAGGATG

      LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
3481  TTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTT
      AACTTTCCGAGGAGCCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCACCCGTATAAA

      ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
3541  AGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAAC
      TCCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG
```

FIG. 1J

FIG. 1K


LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
3601 CTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCCTCTCCACCAGTAGTGCCC
GATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGGTCATCACGGG
GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
3661 CAGAGCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAGCGGCAAAAGCACCAAGGTC
GTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCGCCGTTTTCGTGGTTCCAG
ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
3721 CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTTGCTGCA
GGCCGACGTATACGTCGAGTCCCGATATTCCACGATCATGAGTTGGGGAGACAACGACGT
Leu
ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
3781 ACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACC
TGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCCTGG
GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
3841 GGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTT
CCCCACTCTTGTTAATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAA
AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer
3901 GCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCC
CGGCTGCCGCCCACGAGCCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGG

```
                                  (Val)
      ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
3961  ACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGACCAAGCAGAGACTGCGGGG
      TGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACTGGTTCGTCTCTGACGCCCC

      AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
4021  GCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCCATCCC
      CGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGGTAGGG

      AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
4081  AACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCCTTTTTACGGCAAGGCTATC
      TTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATAG

      ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCys
4141  CCCCTCGAAGTAATCAAGGGGGGGAGACATCTCATCTTCTGTCATTCAAAGAAGAAGTGC
      GGGGAGCTTCATTAGTTCCCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACG

      AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
4201  GACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGT
      CTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCA

      LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu
4261  CTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTC
      GAACTGCACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAG

                                                      Tyr
      MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
4321  ATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTCACCCAG
      TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC
```

FIG. 1L

FIG. 1M

```
                                  (Ser)
     ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
4381 ACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGACAATCACGCTCCCCCAGGAT
     TGTCAGCTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTGTTAGTGCGAGGGGGTCCTA

     AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
4441 GCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGGAAGCCAGGCATCTACAGA
     CGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCCTTCGGTCCGTAGATGTCT

     PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
4501 TTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTCGTCCGTCCTCTGTGAGTGC
     AAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACG

     TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
4561 TATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCCGAGACTACAGTTAGGCTACGA
     ATACTGCGTCCGACACGAACCATACTCGAGTGCGGGCGGCTCTGATGTCAATCCGATGCT

     AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
4621 GCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTTGGGAGGGC
     CGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCCTGGTAGAACTTAAAACCCTCCCG

     ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
4681 GTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGG
     CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCC

     GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
4741 GAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCT
     CTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACGCGATCCCGAGTTCGGGGA
```

```
     ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
4801 CCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCTCAAGCCCACCCTCCATGGG
     GGGGGTAGCACCCTGGTCTACACCTTCACAAACTAAGCGGAGTTCGGGTGGGAGGTACCC

     ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
4861 CCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGAAATCACCCTGACGCACCCA
     GGTTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGT

     ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp
4921 GTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGG
     CAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACC

     ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal
4981 GTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTG
     CACGAGCAACCGCCGCAGGACCGACGAAACCGGCGCATAACGGACAGTTGTCCGACGCAC

     ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal
5041 GTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTC
     CAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAG

     LeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln
5101 CTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAA
     GAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTT
```

FIG. 1N

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FIG. 10

```
      GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer
5161  GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCC
      CCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGGACGTCTGGCGCAGG

      ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe
5221  CGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGGCAAAAACTCGAGACCTTC
      GCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAG

      TrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr
5281  TGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATACTTGGCGGGCTTGTCAACG
      ACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATGAACCGCCCGAACAGTTGC

      LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro
5341  CTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTCACCAGCCCA
      GACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATGTCGACGACAGTGGTCGGGT

      LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu
5401  CTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGGGTGGGTGGCTGCCCAGCTC
      GATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCCACCCACCGACGGGTCGAG

      AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly
5461  GCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGC
      CGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAATCGACCGCGGCGGTAGCCG

      SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla
5521  AGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGGGTATGGCGCGGGCGTGGCG
      TCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCGC
```

```
                                                          (Gly)
     GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal
5581 GGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCCTCCACGGAGGACCTGGTC
     CCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCAG

     AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
5641 AATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCA
     TTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCACCAGACACGTCGT

     IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle
5701 ATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCAGTGGATGAACCGGCTGATA
     TATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGTCACCTACTTGGCCGACTAT

     AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
5761 GCCTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTACGTGCCGGAGAGCGATGCA
     CGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGATGCACGGCCTCTCGCTACGT

                                (HisCys)
     AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
5821 GCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTG
     CGACGGGCGCAGTGACGGTATGAGTCGTCGGAGTGACATTGGGTCGAGGACTCCGCTGAC

     HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
5881 CACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGTTCCTGGCTAAGGGACATC
     GTGGTCACCTATTCGAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG
```

FIG. 1P

FIG. 1Q

```
      TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
5941  TGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATG
      ACCCTGACCTATACGCTCCACAACTCGCTGAAATTCTGGACCGATTTTCGATTCGAGTAC

      ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
6001  CCACAGCTGCCTGGGATCCCCTTTGTGTCCTGCCAGCGCGGGTATAAGGGGGTCTGGCGA
      GGTGTCGACGGACCCTAGGGGAAACACAGGACGGTCGCGCCCATATTCCCCCAGACCGCT

      (Val)
      GlyAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
6061  GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAA
      CACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT

      AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
6121  AACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC
      TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTGTACACCTCACCCTGGAAG

      ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
6181  CCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCTTCCTGCGCCGAACTACACGTTC
      GGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGAAGGACGCGGCTTGATGTGCAAG

      AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
6241  GCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCAC
      CGCGATACCTCCCACAGACGTCTCCTTATACACCTCTATTCCGTCCACCCCCTGAAGGTG

      TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu
6301  TACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCGAA
      ATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTT
```

```
      PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
6361  TTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGCGCCCCCCTGCAAGCCCTTG
      AAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGCGGGGGGACGTTCGGGAAC

      LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu
6421  CTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGGTCGCAATTA
      GACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATGGGCCATCCCAGCGTTAAT

      ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
6481  CCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCAT
      GGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGTGACTAGGGAGGGTA

      IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
6541  ATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGGATCACCCCCCTCTGTGGCCAGC
      TATTGTCGTCTCCGCCGGCCCGCTTCCAACCGCTCCCCTAGTGGGGGGAGACACCGGTCG

      SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
6601  TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCATGAC
      AGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTACTG

      SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
6661  TCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGGCGGCAAC
      AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCCTCTACCCGCCGTTG
```

FIG. 1R

FIG. 1S

```
      IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal
6721  ATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTTGTG
      TAGTGGTCCCAACTCAGTCTTTTGTTTCACCACTAAGACCTGAGGAAGCTAGGCGAACAC

      AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg
6781  GCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATCCTGCGGAAGTCTCGGAGA
      CGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCTTCAGAGCCTCT

      PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr
6841  TTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAACCCCCCGCTAGTGGAGACG
      AAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATTGGGGGGCGATCACCTCTGC

      TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProProLys
6901  TGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAAG
      ACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGTTTC

      SerProProValProProProArgLysLysArgThrValValLeuThrGluSerThrLeu
6961  TCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGTCCTCACTGAATCAACCCTA
      AGGGGAGGACACGGAGGCGGAGCCTTCTTCGCCTGCCACCAGGAGTGACTTAGTTGGGAT

                          (Ser)
      SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle
7021  TCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTTCCGGCATT
      AGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAA

      ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer
7081  ACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCTTCTGGCTGCCCCCCCGACTCC
      TGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGG
```

```
                (PheAla)
      AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu
7141  GACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGAGCCTGGGGATCCGGATCTT
      CTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAA

      SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
7201  AGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGCGGAGGATGTCGTGTGCTGC
      TCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGCCTCCTACAGCACACGACG

      SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys
7261  TCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGCGCCGCGGAAGAACAGAAA
      AGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACGCGGCGCCTTCTTGTCTTT

      LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThr
7321  CTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCACAATTTGGTGTATTCCACC
      GACGGGTAGTTACGTGATTCGTTGAGCAACGATGCAGTGGTGTTAAACCACATAAGGTGG

      ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu
7381  ACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATTTGACAGACTGCAAGTTCTG
      TGGAGTGCGTCACGAACGGTTTCCGTCTTCTTTCAGTGTAAACTGTCTGACGTTCAAGAC

      AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSerLysValLysAla
7441  GACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCGGCGTCAAAAGTGAAGGCT
      CTGTCGGTAATGGTCCTGCATGAGTTCCTCCAATTTCGTCGCCGCAGTTTTCACTTCCGA
```

FIG. 1T

FIG. 1U

```
      (Phe)
      AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys
7501  AACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCCACACTCAGCCAAATCCAAG
      TTGAACGATAGGCATCTCCTTCGAACGTCGGACTGCGGGGGTGTGAGTCGGTTTAGGTTC

      PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn
7561  TTTGGTTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAAGGCCGTAACCCACATCAAC
      AAACCAATACCCCGTTTTCTGCAGGCAACGGTACGGTCTTTCCGGCATTGGGTGTAGTTG

      SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla
7621  TCCGTGTGGAAAGACCTTCTGGAAGACAATGTAACACCAATAGACACTACCATCATGGCT
      AGGCACACCTTTCTGGAAGACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGA

      LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle
7681  AAGAACGAGGTTTTCTGCGTTCAGCCTGAGAAGGGGGGTCGTAAGCCAGCTCGTCTCATC
      TTCTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCCAGCATTCGGTCGAGCAGAGTAG

      ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr
7741  GTGTTCCCCGATCTGGGCGTGCGCGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACA
      CACAAGGGGCTAGACCCGCACGCGCACACGCTTTTCTACCGAAACATGCTGCACCAATGT

      LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg
7801  AAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCAATACTCACCAGGACAGCGG
      TTCGAGGGGAACCGGCACTACCCTTCGAGGATGCCTAAGGTTATGAGTGGTCCTGTCGCC

      ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp
7861  GTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGAT
      CAACTTAAGGAGCACGTTCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA
```

```
      ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr
7921  ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTAC
      TGGGCGACGAAACTGAGGTGTCAGTGACTCTCGCTGTAGGCATGCCTCCTCCGTTAGATG

      GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu
7981  CAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTT
      GTTACAACACTGGAGCTGGGGGTTCGGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAA

                                    (Gly)
      TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
8041  TATGTTGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGC
      ATACAACCCCCGGGAGAATGGTTAAGTTCCCCCCTCTTGACGCCGATAGCGTCCACGGCG

      AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg
8101  GCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCACTTGCTACATCAAGGCCCGG
      CGCTCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGAACGATGTAGTTCCGGGCC

      AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu
8161  GCAGCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA
      CGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAGCACACACCGCTGCTGAAT

      ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr
8221  GTCGTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGGCGAGCCTGAGAGCCTTCACG
      CAGCAATAGACACTTTCGCGCCCCCAGGTCCTCCTGCGCCGCTCGGACTCTCGGAAGTGC
```

FIG. 1V

FIG. 1W

```
     GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu
8281 GAGGCTATGACCAGGTACTCCGCCCCCCCTGGGGACCCCCCACAACCAGAATACGACTTG
     CTCCGATACTGGTCCATGAGGCGGGGGGGACCCCTGGGGGGTGTTGGTCTTATGCTGAAC

     GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg
8341 GAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCACGACGGCGCTGGAAAGAGG
     CTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTGCTGCCGCGACCTTTCTCC

     ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla
8401 GTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGAGCTGCGTGGGAGACAGCA
     CAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCTCGACGCACCCTCTGTCGT

     ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp
8461 AGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATGTTTGCCCCCACACTGTGG
     TCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGGTGTGACACC

     AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu
8521 GCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTATAGCCAGGGACCAGCTTGAA
     CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCGAACTT

     GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro
8581 CAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGAACCACTTGATCTACCT
     GTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTATCTTGGTGAACTAGATGGA

     ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly
8641 CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT
     GGTTAGTAAGTTTCTGAGGTACCGGAGTCGCGTAAAAGTGAGGTGTCAATGAGAGGTCCA
```

```
      GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
8701  GAAATTAATAGGGTGGCCGCATGCCTCAGAAAACTTGGGGTACCGCCCTTGCGAGCTTGG
      CTTTAATTATCCCACCGGCGTACGGAGTCTTTTGAACCCCATGGCGGGAACGCTCGAACC

      Gly
      ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle
8761  AGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATA
      TCTGTGGCCCGGGCCTCGCAGGCGCGATCCGAAGACCGGTCTCCTCCGTCCCGACGGTAT

      CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla
8821  TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCTCAAACTCACTCCAATAGCG
      ACACCGTTCATGGAGAAGTTGACCCGTCATTCTTGTTTCGAGTTTGAGTGAGGTTATCGC

      AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle
8881  GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGGCTACAGCGGGGGAGACATT
      CGGCGACCGGTCGACCTGAACAGGCCGACCAAGTGCCGACCGATGTCGCCCCCTCTGTAA

                                                          (Pro)
      TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuLeuAla
8941  TATCACAGCGTGTCTCATGCCCGGCCCCGCTGGATCTGGTTTTGCCTACTCCTGCTTGCT
      ATAGTGTCGCACAGAGTACGGGCCGGGGCGACCTAGACCAAAACGGATGAGGACGAACGA
```

FIG. 1X

FIG. 1Y

```
      AlaGlyValGlyIleTyrLeuLeuProAsnArgOP
9001  GCAGGGGTAGGCATCTACCTCCTCCCCAACCGATGAAGGTTGGGGTAAACACTCCGGCCT
      CGTCCCCATCCGTAGATGGAGGAGGGGTTGGCTACTTCCAACCCCATTTGTGAGGCCGGA
```

( ) = Heterogeneity due possibly to 5' or 3'-terminal cloning artefact

FIG. 2